

Comm.
Dr. Bing
Dr. Cattell
Dr. Loosli

CHRONIC PULMONARY DISEASES

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N.Y. 10022
(212) 421-8985

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#599 - 4/1/67-3/31/72
CF 436, 7/1/64-4/1/67

FEB 7 1973

Date: January 29, 1973

Application for Research Grant
(Use extra pages as needed)

1. Principal Investigator (give title and degrees).

Domingo M. Aviado, M.D.
Professor of Pharmacology
University of Pennsylvania School of Medicine

2. Institution & address:

University of Pennsylvania School of Medicine
36th Street and Hamilton Walk
Philadelphia, Pa. 19174

3. Department(s) where research will be done or collaboration provided:

Department of Pharmacology
Room 114, Old Medical School Building
University of Pennsylvania School of Medicine

4. Short title of study:

Influence of Cigarette Smoke on Pulmonary Emphysema and Bronchospasm

5. Proposed starting date: July 1, 1973.

6. Estimated time to complete: three (3) years

7. Brief description of specific research aims:

Six objectives are planned. Although most of them will run concurrently for three years, the major emphasis for each six-month period will be one of the following in the order listed:

- (a) To determine the mechanism of bronchospasm observed in mice and rats that have been exposed to cigarette smoke for 5 weeks.
- (b) To continue the investigation of any role of cigarette smoke in the pathogenesis of pulmonary emphysema in rodents and primates.
- (c) To examine the effects of important constituents of cigarette smoke on the bronchonotor, pulmonary vascular, bronchovascular and alveolar structures of the lung. The major constituents that will be studied are nicotine, carbon monoxide, skatole and particulate matter.
- (d) To correlate the experimental induction of pulmonary emphysema or bronchospasm with chemical analysis of the lung, with special emphasis on the composition of phospholipid.
- (e) To correlate functional changes in the lung with histological and ultra-structural examination.
- (f) To develop means of preventing or correcting the abnormalities in function of the lung associated with administration of cigarette smoke or its constituents.

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2.
8. Brief statement of working hypothesis:

Since 1962, the principal investigator has been studying the acute effects of cigarette smoke on dogs, cats, rabbits, hamsters, rats and mice and its chronic effects on the last two species. The results indicate that the bronchomotor system is more sensitive than the pulmonary and bronchial blood vessels to inhalation of cigarette smoke. In the prolonged and repeated administration of cigarette smoke to rats and mice for 5 weeks, there are no signs of pulmonary emphysema and only bronchospasm persists as the most significant functional abnormality. Before discounting the role of cigarette smoking in the pathogenesis of pulmonary emphysema, it is planned to prolong the exposure to cigarette smoke from 5 weeks to 6 months in rodents and to include primates. Since cigarette smoking has been suspected of causing pulmonary emphysema and increase in airway resistance in man, the lesions should be reproducible in animals. It also follows that the mode of action of cigarette smoke on the pulmonary structures can properly be identified in animals. This is our working hypothesis which will be tested during the next 3 years.

9. Details of experimental design and procedures (append extra pages as necessary)

(a) Mechanism of bronchospasm following chronic exposure to cigarette smoke.

In 1968, Ito and Aviado (1) reported a technique for development of experimental emphysema in the rat, which could be used to test agents for provoking or preventing the pulmonary lesions. Although measurements of functional residual capacity did not reveal emphysematous lesions from chronic exposure to cigarette smoke, there was an increase in pulmonary resistance, indicating bronchospasm. During the past year techniques for measuring functional residual capacity, pulmonary resistance and pulmonary compliance were developed in this laboratory (see Progress Report submitted January 31, 1973). Six weeks' exposure to cigarette smoke did not induce pulmonary emphysema but resulted in bronchospasm. Our plan is to determine the mechanism for the development of bronchospasm by testing the influence of parasympathetic blocking agents (such as atropine), ganglionic blocking drugs (such as chlorisondamine) and antihistaminic drugs (such as chlorpheniramine), because previously completed experiments show that in most animal species the short-term inhalation of cigarette smoke stimulated cholinergic and histaminergic receptors in the lung (2). If the concurrent use of blocking agents is unsuccessful, then other mechanisms will be considered, such as release of other bronchospastic humoral agents and structural changes in mucous glands and smooth muscles of the airways. The morphology of the airways will be examined in detail under (e) below.

(b) Pulmonary emphysema in rodents and primates. The long-term exposure of mice and rats will be extended to 6 months. Pulmonary emphysema will be assessed by measurement of functional residual capacity, described under (a) above, and by histological examination, described under (e) below. The details of producing experimental pulmonary emphysema have not been decided. There are no technical difficulties in measurement of functional resistance or pulmonary resistance and compliance, since the techniques used in dogs and cats are applicable. The major problem will be induction of emphysema and we intend to try initially intratracheal ligation and intratracheal injection of papain, which were successfully applied to rodents in inducing emphysema. Our plan is to use 30 monkeys, 150 rats and 300 mice, half of them as controls and the other half to be subjected to daily inhalation of cigarette smoke. The cigarette machine provided by the Council will be used for mice and rats and we plan to develop a special one for monkeys.

(c) Constituents of cigarette smoke. Our previously reported experiments included the testing of acute administration of nicotine (3). We plan to administer nicotine in mist form chronically to mice, rats and monkeys. Since skatole has

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9. Details of experimental design and procedures (Continued)

been reported by Carlson *et al.* (9) to produce pulmonary emphysema in cattle and since it is also a constituent of cigarette smoke, this substance will be administered either orally or by inhalation in mice and rats to determine development of bronchospasm and pulmonary emphysema. Carbon monoxide is the third constituent of cigarette smoke that will be tested in mice and rats for chronic action on airways and airspaces. Any long-term effects of nicotine, skatole and carbon monoxide will be investigated for their mode of action in a manner described under section (a) above.

(d) Chemical analysis of lungs and blood. This laboratory has reported techniques for analysis of biogenic amines in the lungs (4) and individual phospholipids (see Progress Report submitted January 31, 1973). The amines relate to the mechanism of bronchospasm and would supplement the observations planned under (a) above. The phospholipids are the basis for surfactant activity of lung extract and may appear concurrently with a reduction in pulmonary compliance, which is likely to occur when the lungs develop pulmonary congestion and edema. Blood analysis for antitrypsin activity, carboxyhemoglobin saturation and gas tensions will be performed by the standard techniques. These measurements will be performed in conjunction with the assessment of lung function described above under (a), (b) and (c).

(e) Histological and ultrastructural examination of pulmonary tissue. The technique described by Loosli *et al.* (10) for removal and fixation of the lungs has been adopted. The estimation of airspaces relative to alveolar walls on a grid will continue. In addition, the principal investigator will collaborate with a pathologist, who will examine the tissue slices by microscope and electron microscope. In conjunction with experiments described in sections (a), (b) and (c), the microscopic examination will allow the localization of early onset of lesions in the airways, blood vessels and alveolar walls. The histochemical technique for identification of fluorescent substances (such as serotonin and catecholamines) developed by Jacobowitz and Koelle (11) will also be applied to the lungs that have been removed from the animals exposed to cigarette smoke and its constituents.

(f) Prevention and correction of abnormalities in lung function. This last aspect of the investigation will be conducted during the final 6 months of the 3-year study. By then, the chronic effects of cigarette smoke will have been identified. In the past, the following drugs have been used either to prevent or to reverse pulmonary malfunction: progesterone against emphysema (5), bronchodilators against bronchospasm (6), naphthoquinones against pulmonary edema (7), and expectorants against deficiency of surfactant (8). Other types of desired actions will be introduced as the pathological processes relating to cigarette smoking are identified.

References Cited Above:

1. Ito, H. and Aviado, D. M.: Pulmonary emphysema and cigarette smoke; experimental induction and use of bronchodilators in rats. Arch. Environ. Health 16:865-870, 1968.
2. Aviado, D. M. and Palecek, F.: Pulmonary effects of tobacco and related substances; I, II, III: Arch. Environ. Health 15:187-213, 1967.
3. Aviado, D. M. and Samarek, M.: Bronchopulmonary effects of tobacco and related substances; I, II, III, IV: Arch. Environ. Health 11:141-176, 1965.

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References (Continued):

4. Sadavongvivad, C. and Aviado, D. M.: Pharmacological significance of biogenic amines in the lungs; I, II, III: Brit. J. Pharmacol. 38:353-385, 1970.
5. Inoh, T. and Aviado, D. M.: Cardiopulmonary effects of progestational agents in emphysematous rats. Chest 59:659-666, 1971.
6. Aviado, D. M., Sadavongvivad, C. and Carrillo, L. R.: Cigarette smoke and pulmonary emphysema; influence of bronchodilators and biogenic amines in experimental induction in rats. Arch. Environ. Health 20:483-487, 1970.
7. Aviado, D. M. and Cambar, P. J.: Pulmonary edema and naphthoquinones. Exper. Parasitology 26:354-368, 1969.
8. Cambar, P. J. and Aviado, D. M.: Bronchopulmonary effects of paraquat and expectorants. Arch. Environ. Health 20:488-494, 1970.
9. Carlson, J. R., Dyer, H. A. and Johnson, R. J.: Tryptophan-induced interstitial pulmonary emphysema in cattle. J. Vet. Research 29:1983-1989, 1968.
10. Loosli, C. G., Hartweck, M. S. and Hockwald, R. S.: Airborne influenza PR8-A virus infections in actively immunized mice. Arch. Environ. Health 21:332-346, 1970.
11. Jacobowitz, D. and Koelle, G. B.: Histochemical correlations of acetylcholinesterase and catecholamines in postganglionic autonomic nerves of the cat, rabbit and guinea pig. J. Pharmacol. Exper. Ther. 148:225-237, 1965.

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

Laboratories totaling 1,600 square feet are available for this investigation. They contain the following equipment:

- (a) Polygraph and oscilloscopes for measurement of pulmonary resistance and compliance, blood pressures and heart rate; and also artificial respiratory and perfusion pumps.
- (b) Spectrophotometer and equipment for paper chromatography.
- (c) Gas chromatography apparatus, tonometer for measurement of partial pressure, and Scholander gas analyser.
- (d) Fluoroscope for insertion of catheter into cardiopulmonary area.
- (e) Microscopes and apparatus for tissue fixation, slicing and staining.
- (f) Animal cages.
- (g) Special smoking equipment supplied by the Council for Tobacco Research.

11. Additional facilities required:

Special chambers for administering carbon monoxide, cigarette smoke or aerosol mist from a solution of nicotine or skatole.

12. Biographical sketches of investigator(s) and other professional personnel (append).

13. Publications (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

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12. Biographical sketches of investigator(s) and other professional personnel:

During the first year, Drs. Belej and Watanabe will continue to collaborate with the principal investigator. During the second and third years, a pathologist and a biochemist will collaborate with him. They will be selected at a future date.

13. Publications:

Aviado, D. M. and Palecek, F.: Pulmonary effects of tobacco and related substances; I, II, III: Arch. Environ. Health 15:187-213, 1967.

Ito, H. and Aviado, D. M.: Pulmonary emphysema and cigarette smoke; experimental induction and use of bronchodilators in rats. Arch. Environ. Health 16: 865-870, 1968.

Aviado, D. M., Sadavongvivad, C. and Carrillo, L. R.: Cigarette smoke and pulmonary emphysema; influence of bronchodilators and biogenic amines in experimental induction in rats. Arch. Environ. Health 20:483-487, 1970.

Aviado, D. M. and Sadavongvivad, C.: Pharmacological significance of biogenic amines in the lungs: 5-hydroxytryptamine, histamine, noradrenaline and dopamine. Brit. J. Pharmacol. 38:353-385, 1970.

Inoh, T. and Aviado, D. M.: Cardiopulmonary effects of progestational agents in emphysematous rats. Chest 59:659-666, 1971.

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CURRICULUM VITAE OF DOMINGO M. AVIADO

Born

REDACTED

College Education:

University of the Philippines College of Liberal Arts	1940-1942
University of the Philippines College of Medicine	1942-1945
University of Pennsylvania School of Medicine	1946-1948
Doctor of Medicine, University of Pennsylvania	March 1948

Academic Positions at the University of Pennsylvania:

Assistant Instructor in Pharmacology	1948-1949
Instructor in Pharmacology	1949-1950
Associate in Pharmacology	1950-1953
Assistant Professor of Pharmacology	1953-1960
Associate Professor of Pharmacology	1960-1965
Professor of Pharmacology	1965-pres.
Member, Parasitology Graduate Group	1967-pres.

Miscellaneous Positions and Honors:

National Institute of Health Post-Doctorate Research Fellow	1948-1950
Section Editor of Chemical Abstracts	1952-1958
Assistant Attending Physician of Cardiology, Philadelphia General Hospital	1955-pres.
Visiting Lecturer in Anesthesiology, Albert Einstein Medical Center	1955-pres.
Associate Editor of Circulation Research	1958-1962
Visiting Lecturer in Pharmacology, University the East, R. M. M. C.	1959
Visiting Lecturer in Physiology, Women's Medical College	1961-1962
Travel Award Rockefeller Foundation	1961
Linnaeus Medal, First International Pharmacological Meeting, Stockholm	1961
Fellow of the Guggenheim Foundation	1962-1963
Purkinje Medal, Second International Pharmacological Meeting, Prague	1963
Editorial Consultant, Dorland' Illustrated Medical Dictionary	1963-1967
Consultant, Poison Control Program of Philadelphia	1964-pres.
Visiting Lecturer in Physiology, Rutgers University	1966-1967
Ad Hoc Committee on Air Pollution and Air Hygiene, Philadelphia Medical Society, Member	1967-1969
Physician of the Year Award, Philippine Medical Association (Chicago)	1969
Member, Bronchopulmonary Panel, National Clearinghouse for Smoking and Health	1969
Chairman, Medical Advisory Committee, Clinical Research Center, Graduate Hospital of University of Pennsylvania	1969-1970
Member, American Heart Association Ad Hoc Committee on Cigarette Smoking	1969-1970
Editor, "Scapel and Tongs" (Medical Stamps Monthly)	1971-

Societies:

Alpha Omega Alpha Honorary Medical Society: Member	1946
Physiological Society of Philadelphia: Member, 1948, Secretary, 1954-1958; President, 1959-1960; Councillor	1960-1961

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American Society for Pharmacology and Experimental Therapeutics:	
Member 1950: Co-Chairman, 1965 Fall Meeting, Member	
Finance Committee	1965-1970
American Physiological Society: Member	1951-
American Association for the Advancement of Science	1951
The Society of Sigma XI: Member	1952
John Morgan Society of the University of Pennsylvania, Member	
1956, Life member, 1967	
American Heart Association: Member 1957; Member Research Study	
Committee 1965-1967	
Section on Pharmacology (SEPHAR); International Union of Physiological	
Sciences-Treasurer	1959-1965
International Union of Pharmacology (IUPHAR) Treasurer	1965-1966
American Society of Tropical Medicine and Hygiene: Member	1966
International Leprosy Association: Member	1967
American College of Clinical Pharmacology: Charter Member:	1971

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R: REDACTED MATERIAL

CURRICULUM VITAE

MIROSŁAW A. BELEJ

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Date of birth:

REDACTED

Field Pharmacology

Degree Ph. D. (June 1971)

Education

1969-1971 THOMAS JEFFERSON UNIVERSITY MEDICAL COLLEGE

Received Ph. D. degree in June 1971. Pharmacology major.
Courses taken: Pharmacology, Toxicology, Drug Metabolism,
Principles of Drug Action.

1965-1968 TEMPLE UNIVERSITY MEDICAL SCHOOL

Received M. S. degree in June 1968. Majored in Pharmacology;
courses included gross anatomy, embryology, histology,
neuroanatomy, physiology, biochemistry, pathology, electro-
physiology, flow dynamics and biostatistics.
Research in cardiovascular area. M. S. thesis published.

1960-1964 TEMPLE UNIVERSITY COLLEGE OF LIBERAL ARTS

Received B. A. degree in February 1964. Biology major.
Among courses taken were mammalian anatomy, comparative
anatomy, animal embryology, histology, physiology,
bacteriology, organic chemistry, qualitative and quantitative
chemistry, and calculus.

Training and
Experience

1971-1972

REDACTED

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Miroslaw A. Belej

1969-1971

1968-1969

REDACTEDForeign Languages

Speak, read and write: French and Ukrainian
Read: Russian

Societies**REDACTED**References

Dr. D. M. Aviado, Professor of Pharmacology
University of Pennsylvania
Department of Pharmacology
Philadelphia, Pa. 19104 Tel: (215) 594-8413

Dr. R. W. Manthei, Professor of Pharmacology
Thomas Jefferson University
Jefferson Medical College
Department of Pharmacology
Philadelphia, Pa. 19107 Tel: (215) 829-7969

Dr. J. M. Coon, Professor of Pharmacology
and Chairman of the Department
Thomas Jefferson University
Jefferson Medical Collge
Philadelphia, Pa. 19107 Tel: (215) 829-7766

Publications

Belej, M. A. et al. The Mechanism of "Nicotine Reversal"
in Phenoxybenzamine-treated Dogs. J. Pharmacol.
Exp. Ther. 164: 342-347, 1968.

Aviado, D. M. and Belej, M. Pharmacology of New Antimalarial
Drugs: Two Quinolinemethanols. Pharmacology 3: 257-
272, 1970

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CURRICULUM VITAE

TETSUYA WATANABE

REDACTEDDate of birth: **REDACTED** **REDACTED**Degree D. D. S. (1970)Education

1964-1967 Pre dental courses taken in Yokohama National University and Kanagawa Dental College.

1967-1971 Received D. D. S. degree on March, 1971 from Kanagawa Dental College. Passed National Board in Dental Medicine on May 1971.

Training and Experienced1971 (April-Sept.) **REDACTED**1971-1972 **REDACTED**SocietiesPublications

Watanabe, T. and Ito, H. Determination of Inorganic phosphate in presence of G-1-P and Phosphorylase activity. To be submitted for publication.

Watanabe, T. and Ito, H. Quantitative Determination of Phosphorylase Activity in Rat After Disc Electrophoresis on Polyacrylamide Gel and Changes of It's Acitivity due to the Electrophoresis. Biochim. Biophysica Acta. Submitted for publication.

Watanabe, T., Ito, H. and Cho, Y. Effects of Thyroxine and ADR on cardiac muscle (phosphorylase activity and nucleotides) To be submitted for publication.

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R: REDACTED MATERIAL

14. First year budget:

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
Domingo H. Aviado, M.D. Professor of Pharmacology	30%	R
Miraslaw A. Belej, Ph.D. Assistant Professor of Pharmacology	60%	
Tetsuya Watanabe, D.D.S. Instructor in Pharmacology	100%	

Technical

Henry A. Reutter, Technician	30%	R
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Employee benefits (15% of professional and
8% of technical)

Sub Total for A \$25,160

B. Consumable supplies (by major categories)

Animals and animal care	5,000
Chemicals and glassware	1,000

Sub-Total for B \$ 6,000

C. Other expenses (itemize)

Reprints and publication costs	500
Travel to attend national meetings	500

Sub-Total for C \$ 1,000

Running Total of A + B + C \$32,160

D. Permanent equipment (itemize)

Chamber for exposure of animals	2,000
Transducers	1,000

Sub-Total for D \$ 3,000

E \$ 4,824

Total request \$39,984

E. Indirect costs (15% of A+B+C):

15. Estimated future requirements.

	Salaries	Consumable Suppl	Other Expenses	Permanent Equip	Indirect Costs	Total
Year 2	25,160	6,000	1,000	3,000	4,824	\$39,984
Year 3	25,160	6,000	1,000	3,000	4,824	\$39,984

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16. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Bronchovascular Effects of Cigarette Smoke	Council for Tobacco Research (CTR Grant 599)	\$36,775	July 1, 1972 - June 30, 1973
Drug Therapy of Acute Respiratory Insufficiency	Department of the Army (DADA 17-71-C-1060)	\$29,407	April 1, 1972 - March 31, 1973
Cardiopulmonary Toxicity of Propellants for Aerosols	Food and Drug Administration (FDA71-310)	\$54,000	September 1, 1972 - August 31, 1973

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Drug Therapy of Acute Respiratory Insufficiency	Department of the Army	\$37,053	April 1, 1973 - March 31, 1974

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Domingo H. Aviado, M.D.Signature Domingo H. Aviado Date 1/29/73Telephone (215) 594-8413

Area Code _____ Number _____ Extension _____

Checks payable to

Mailing address for checks

Responsible officer of institution

Typed Name _____

Title _____

Signature _____ Date _____

Telephone _____

Area Code _____ Number _____ Extension _____

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